

**IN THE CLAIMS:**

1-32. (Canceled)

33. (Currently amended) A method of silencing a target gene in an organism by post-transcriptional gene silencing (PTGS), the method comprising the step of introducing into the organism a silencing agent ~~which base pairs with said target gene,~~

~~wherein the silencing agent comprises~~ comprising short RNA molecules (SRMs) comprising consisting of SARMs which hybridize to sense strands of the target gene or mRNA and corresponding complementary ~~corresponding complementary SSRMs which could hybridize to antisense strands of the target gene, each of said SARMs and SSRMs being which are 25 nucleotides (25nt) in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which are specific for the targeted region of the~~ and being effective to silence the target gene or mRNA when present in a cell expressing said target gene.

34. (Cancelled)

35. (Cancelled)

36. (Previously presented) The method of claim 33 wherein said silencing agent consists of SRMs which are 21 to 24 ~~25~~ nucleotides in length.

Claims 37-39 (Cancelled)

40. (Currently amended) A method of silencing a target gene in an organism as claimed in claim 33, wherein the step of introducing said silencing agent comprising comprises

(a) providing a DNA construct containing a promoter operably linked to a DNA which upon transcription in a host cell results in expression of a 20-254nt silencing agent specific to a target gene, wherein the silencing agent comprises one or more short RNA molecules (SRMs) which are 25 nucleotides in length ~~plus or~~ minus 1, 2, 3, 4 or 5 nucleotides, wherein said SRMs comprise SARMs which hybridize to sense strands or mRNA of the target gene and corresponding complementary SSRMs which could hybridize to antisense strands of the target gene,

(b) introducing said construct into said organism such that the target gene in the organism is silenced by the silencing agent transcribed by said promoter.

41. (Currently amended) The method of claim 40 wherein said silencing agent consists of SRMs 21 to 254 nucleotides in length.

Claims 42-44 (Cancelled)

45. (Withdrawn) A host cell containing a DNA construct which comprises a promoter operably linked to DNA which upon transcription in the host cell results in a silencing agent specific to a target gene, and wherein the silencing agent comprises one or more short RNA molecules (SRMs) which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which SRMs are specific for a targeted region in a target gene and upon transcription

silence the target gene.

46. (Withdrawn) A method of selecting a target region in a target gene which is desired to be silenced comprising:

(I) isolating one or more RNA molecules from a sample, wherein said RNA molecules are short RNA molecules (SRMs) which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and which are specific for a target region of a target gene by:

(a) producing a nucleic acid extract from said sample;

(b) purifying said extract to obtain purified RNA molecules by effecting at least one purification step selected from the group consisting of (i) filtration; (ii) differential precipitation and (iii) ion exchange chromatography and isolating SRMs which are silencing agents for said target gene;

(II) identifying a target region in the sequence of said target gene which corresponds to a sequence comprised in said SRMs.

47. (Withdrawn) The method of claim 46 which further comprises separating the purified RNA molecules according to size by gel electrophoresis using a 15% polyacrylamide, gel containing 7M urea as a denaturant and TBE (0.5x) as a buffer.

48. (Withdrawn) The method of claim 47 which further comprises transferring the RNA molecules comprised on the gel to a hybridization membrane by electrophoresis.

49. (Withdrawn) The method of claim 48 which further

comprises labeling the RNA molecules comprised on the hybridization membrane using a radioactive probe obtained from a single stranded RNA molecule transcribed in vitro from a plasmid DNA template.

50. (Withdrawn) A method of silencing a target gene in an organism comprising:

- (i) performing a method according to claim 45 to select a target region of a target gene to be silenced; and
- (ii) silencing said target gene in an organism by targeting said target region with a silencing agent.

51. (Withdrawn) The method of claim 50 wherein step (ii) is effected by introducing into the organism SRMs specific to the targeted region of the target gene which induce silencing of said target gene.

52. (Withdrawn) The method of claim 51 wherein said SRMs comprise RNA molecules which are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides.

53. (Withdrawn) The method of claim 52 wherein said SRMs comprise RNAs which are 21 to 25 nucleotides in length.

54. (Withdrawn) A method of silencing a target gene in a first organism comprising:

- (i) generating in a second organism short RNA molecules (SRMs) which are a silencing agent for said target gene, wherein said SRMs are 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides, and are specific for a target region in said target gene; and
- (ii) introducing said SRMs into said first organism in

order to silence said target gene comprised therein.

55. (Withdrawn) The method of claim 54 wherein said SRMs are 21 to 25 nucleotides in length.

56. (Withdrawn) The method of claim 54 wherein said SRMs are short anti-sense RNA molecules (SARMs) and/or short sense RNA molecules (SSRMs).

57. (Withdrawn) The method of claim 54 wherein said SRMs are SARMs.

58. (Withdrawn) The method of claim 54 wherein said SRMs are SSRMs.

59. (Withdrawn) The method of claim 54 wherein said target gene is endogenous to the first organism but is not endogenous to the second organism.

60. (Currently amended) A method of inhibiting the translation of a gene product in a cell by post-transcriptional gene silencing by introducing into said cell ~~at least one~~ short RNA molecules (SRMs) ~~that is~~ 25 nucleotides in length ~~plus or~~ minus 1, 2, 3, 4 or 5 nucleotides, wherein said SRMs comprises SARMs which hybridize to sense strands of the gene or mRNA and corresponding complementary SSRMs which could hybridize to antisense strands of the gene, wherein said SARM has a sequence complementary to an mRNA that encodes said gene product.

61. (Currently amended) The method of Claim 60 wherein

said SRM is 20 to 24 ~~25~~ nucleotides in length.

62. (Previously presented) The method of Claim 60 wherein said SRM is 21 to 24 nucleotides in length.

63. (Previously presented) The method of Claim 60 wherein said SRM is 21 to 23 nucleotides in length.

64. (Previously presented) The method of Claim 60 wherein said SRM does not affect transcription of mRNA encoding said gene product.

65. (Previously presented) The method of Claim 60 wherein said SRM is the only exogenously introduced RNA that inhibits the translation of said gene product.

66. (Previously presented) The method of Claim 60 wherein said introduced SRM is transcribed from a DNA vector or construct introduced into said cell.

67. (Previously presented) The method of Claim 66 wherein said vector or construct is stably maintained by said cell.

68. (Previously presented) The method of Claim 67 wherein said vector or construct transcribes one or more SRMs.

69. (Previously presented) The method of Claim 60 wherein said cell is from an organism selected from the group consisting of a plant, mammal, insect, avian, reptile, protozoan and nematode.

70. (Currently amended) The method of Claim 69 wherein said

cell is comprised in a human or non-human organism.

71. (Previously presented) The method of Claim 60 wherein said SRM silences a target gene in said cell.

72. (Previously presented) The method of Claim 71 wherein said target gene is endogenous to said cell.

73. (Previously presented) The method of Claim 71 wherein said target gene is selected from the group consisting of a gene involved in cancer, apoptosis, cell-cycle regulation, a neurological process and signal transduction.

74. (Previously presented) The method of Claim 71 wherein said target gene is selected from the group consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member gene.

75. (Currently amended) The method of Claim 71 wherein said target gene is expressed by a virus, parasite or predator contained within said cell ~~of said organism~~.

76. (Previously presented) The method of Claim 71 wherein said target gene is involved in parasite resistance.

77. (Cancelled)

78. (Cancelled)

79. (Previously presented) The method of Claim 60 wherein said cell is comprised in an organism.

80. (Previously presented) The method of Claim 79 wherein said organism is selected from the group consisting of a plant, mammal, avian, reptile, insect, protozoan, and a nematode.

81. (Withdrawn) The method of Claim 80 wherein said organism is a mammal.

82. (Withdrawn) The method of Claim 81 wherein said organism is a rodent.

83. (Previously presented) The method of Claim 80 wherein said organism is a plant.

84. (Withdrawn) A method of selectively silencing a target gene in a cell comprising introducing an exogenous nucleic acid into said cell, wherein said exogenous nucleic acid comprises:

(a) a transcribable nucleic acid construct encoding a SRM or a precursor of a SRM, wherein said SRM is characterized as a short RNA molecule 25 nucleotides in length plus or minus 1, 2, 3, 4 or 5 nucleotides and which has a nucleic acid sequence complementary to a portion of said target gene; and

(b) a transcriptional promoter upstream from said sequence encoding said SRM, wherein said promoter is selectively only active in a specific cell type, or is only active in a specific cell type, or is only active in response to an externally controllable stimulus.

85. (Withdrawn) The method of Claim 84 wherein said cell is selected from the group consisting of plant, mammalian,



insect, nematode, avian, reptilian, and nematode cell.

86. (Withdrawn) The method of Claim 84 wherein the target gene is endogenous to said cell.

87. (Withdrawn) The method of claim 84 wherein said target gene is selected from the group consisting of genes involved in cancer, apoptosis, cell-cycle regulation, neurological processes and signal transduction.

88. (Withdrawn) The method of Claim 84 wherein said target gene is selected from the group consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member genes.

89. (Withdrawn) The method of Claim 84 wherein said target gene is expressed by a virus, parasite or predator that affects an organism which contains said cell.

90. (Withdrawn) The method of Claim 84 wherein said SRM is transcribed by a vector or construct stably integrated into said cell.

91. (Withdrawn) The method of claim 84 wherein said at least one SRM comprises sense and antisense RNA molecules which are complementary to a portion of said target gene.

92. (Withdrawn) The method of claim 91 wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.

93. (Currently amended) A method of introducing systemic

PTGS of a target gene in an organism which comprises introducing into said organism an SRM or a transcribable nucleic acid construct encoding a SRM wherein said SRM ~~comprises~~ consists of corresponding complementary short RNA molecules 25 nucleotides in length, plus or minus 1, 2, 3, 4 or 5 nucleotides, said SRM comprising SARMs which hybridize to sense strands of the target gene or mRNA and SSRMs which could hybridize to antisense strands of said target gene and which has a nucleic acid sequence complementary to a portion of said target gene and wherein said SRMs comprise SARMs and corresponding complementary SSRMs.

94. (Previously presented) The method of Claim 93 wherein said target gene is endogenous to said cell.

95. (Previously presented) The method of Claim 93 wherein said target gene is selected from the group consisting of a gene involved in cancer, apoptosis, cell-cycle regulation, a neurological process and signal transduction.

96. (Previously presented) The method of Claim 93 wherein said gene is selected from group the consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member gene.

97. (Currently amended) The method of Claim 93 wherein said target gene is expressed by a virus, parasite or predator contained with a ~~said~~ cell of said organism.

98. (Previously presented) The method of Claim 93 wherein said cell is selected from the group consisting of a plant,

mammalian, insect, nematode, avian, reptilian, and protozoan cell.

99. (Previously presented) The method of Claim 93 wherein said SRM is transcribed by a vector or DNA construct introduced into said cell.

100. (Cancelled)

101. (Currently amended) The method of claim 93 ~~100~~ wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.

102. (Currently amended) A method of inducing post transcriptional gene silencing in a cell of an organism in vivo which comprises introducing into said cell a selected nucleic acid sequence wherein said nucleic acid is selected for introduction into said cell of said organism in vivo based on a finding that said nucleic acid when introduced into a cell of said organism in vitro induces the production in said cell of short RNA molecules, which are 25 nucleotides in length, ~~plus or~~ minus 1, 2, 3, 4 or 5 nucleotides, wherein said SRMs comprise SARMS which hybridize to sense strands of the selected nucleic acid and ~~corresponding complementary~~ SSRMs which could hybridize to antisense strands of the selected nucleic acid such that and wherein said nucleic acid is sufficiently complementary in sequence specificity to a mRNA present in said cell in vivo to interfere with the stability and translation of said mRNA in said cell is reduced.

103. (Previously presented) The method of Claim 102 wherein

said SRMs are transcribed by a vector or construct stably integrated into said cell.

104. (Previously presented) The method of Claim 102 wherein said cell is selected from the group consisting of a plant, a mammal, an avian organism, a reptile, an insect, a nematode, and a protozoan cell.

105. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a target gene endogenous to said cell.

106. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a gene selected from the group consisting of a gene involved in cancer, apoptosis, cell-cycle regulation, neurological processes and signal transduction.

107. (Previously presented) The method of Claim 102 wherein said mRNA is transcribed by a gene selected from the group consisting of an oncogene, a transcriptional regulator, a pocket protein and a MHC superfamily member gene.

108. (Withdrawn) The method of Claim 102 wherein said mRNA is transcribed by a gene of a parasite or predator contained within said cell of said organism.

109. (Cancelled)

110. (Currently amended) The method of claim ~~109~~ 102 wherein said sense and antisense RNA molecules are present in essentially equimolar amounts.

111. (Previously presented) The method of claim 33, wherein said SSRMs and said SARMs are present in equal abundance.

112. (Previously presented) The method of claim 40, wherein said SSRMs and said SARMs are present in equal abundance.

113. (Previously presented) The method of claim 60, wherein said SSRMs and said SARMs are present in equal abundance.

114. (Previously presented) The method of claim 93, wherein said SSRMs and said SARMs are present in equal abundance.

115. (Previously presented) The method of claim 102, wherein said SSRMs and said SARMs are present in equal abundance.